IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Weiss *et al.*
Serial No.	:	08/486,313
Filed	:	June 7, 1995
For	:	MULTIPOTENT NEURAL STEM CELL COMPOSITIONS
Examiner	:	A-M. Baker
Group Art Unit	:	1632

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

I, Nobuko Uchida, hereby declare and state as follows:

1. I received my B.A. degree in molecular biology from Wellesley College in 1983. I received my Ph.D. in cancer biology from Stanford University in 1992. I have been working in the field of stem cell biology since 1992, and working with neural stem cells since 1998.

2. I understand that the pending claims are directed to methods for transplanting neural stem progeny into a host.

3. I am aware of the Examiner's January 31, 2001 Final Office Action. In particular, I understand that the Examiner has rejected the pending claims under 35 U.S.C. §112 contending that "[t]he claims are not enabled because the transplantation of multipotent neural stem cell progeny into a host has not been demonstrated to provide any therapeutic benefit to the host."

4. I make this declaration to rebut the Examiner's rejection, with which I do not agree. In view of the express statements in the specification regarding transplantation of neural stem cell cultures and the voluminous experimental evidence that has been accumulated, in my opinion, the ordinarily skilled artisan would be able to routinely transplant the described neural stem cell cultures with a reasonable expectation of success. I am also of the opinion that although no

therapeutic benefit is recited in the claims, that transplantation of neural stems into a host has been clearly demonstrated to confer a therapeutic benefit, and that the ordinarily skilled artisan would believe that such transplantation would provide a therapeutic benefit to the host.

5. Prior to this invention, the operating dogma in neurobiology was that the brain was relatively quiescent, and that there was no "stem cell" that could be proliferated and then differentiated to form the three major cell types in the central nervous system (*i.e.*, neurons, astrocytes and oligodendrocytes). The neural stem cells described for the first time in this invention, and the ability for the art (provided by the inventors here) to obtain proliferating cultures of those cells, has been widely hailed as a landmark in neurobiology. In fact, one of the early publications that describe this invention (Reynolds and Weiss, Science 255:1707-10 (1992)), over 500 published references have cited to this seminal work. A copy of the search results demonstrating this is attached as Ex. 1. A number of these publications demonstrate that a therapeutic benefit is conferred when neural stem cells are transplanted into a host. I will discuss these in detail below.

6. As a general matter, it is my view, based on my knowledge of the field and based on the voluminous citations to the inventors' work, that researchers of ordinary skill in the relevant arts clearly recognized the importance of the Applicants' invention and relied upon it in their subsequent work. In my opinion, the Applicants' invention discloses paradigm-shifting technology and is a vitally important finding in the field of neurobiology. I am of the firm view that the claimed methods for transplanting neural stem cells are enabled, and provide my detailed thoughts below.

7. The instant specification expressly states that the neural stem cell cultures of this invention are particularly suited for transplantation, since, until now, cultures of proliferating neural cells have not been available to the art. Further, with the invention, the tissue source is well-defined, reproducible, and is not derived from an oncogene-immortalized cell line (thus being non-tumorigenic). *See,* specification, pg. 11, lines 15-20. In fact, one of the named

inventors, Dr. Baetge, has concluded that such neural stem cell cultures are "ideal" for nervous system transplantation. *See, Baetge et al.*, 695 Ann N.Y. Acad. Sci., pp. 285-291 (1993). Multiple other workers in the field have reached the same conclusion.

8. Applicants also expressly provided ample guidance in the specification about how to transplant CNS neural stem cells (*see,* specification, pg. 36, line 10, to pg. 42, line 13; pg. 68, line 16, to pg. 69, line 18; pg. 78, line 17, to pg. 79, line 6; pg. 96, line 12, to pg. 97, line 28). Applicants even provided working examples of neural stem cell transplantation in various disease models, including, *e.g.*, Huntington's disease, Parkinson's disease, and cardiac arrest. *See, e.g.*, specification, pp. 96-101. By way of further example, the instant specification teaches and discloses the types of diseases to which the invention is directed. (*See* pg. 40, lines 9-18). The specification provides exemplary teaching of where to transplant the cells of the claimed invention. (*See, e.g.*, pg. 38, lines 17-30). Further, the specification teaches and discloses how to monitor the transplanted cells. (*See* pg. 39, lines 16-31). Additionally, the specification teaches and discloses how to get the transplanted cells to proliferate *in vivo*. (*See* pg. 42, line 14 through page 47, line 26). In my view, it cannot be disputed that the ordinarily skilled artisan, with the specification in hand, would be able to transplant neural stem cell cultures into a host; that is, the ordinarily skilled artisan would know how to use the invention as claimed. I do not believe that the Examiner disputes this; rather I believe that the Examiner is asking for additional proof that the ordinarily skilled artisan would believe that making such a transplantation would confer a therapeutic benefit. I have provided that proof below.

9. The specification contains detailed teachings relating to how to practice the invention, as well as providing forty-five (45) *in vitro* and *in vivo* examples relating to generation and use of neural stem cell cultures. Among these examples are various standard, well-accepted animal models of various human diseases, including, *e.g.*, animal models for Parkinson's disease and Huntington's disease. Applicants also disclosed treatment of neurodegenerative disease using progeny of human neural stem cells proliferated *in vitro*; remyelination in myelin deficient rats using neural stem cell progeny proliferated *in vitro*; remyelination in human neuromyelitis

optica; and remyelination human Pelizaeus-Merzbacher disease. (*See* Specification, Examples 14-17).

10. I note that (1) that the transplanted neural stem cell cultures secrete cellular products which are capable of providing a therapeutic benefit to the host, and (2) that the neural stem cell cultures exhibit tissue-specific differentiation upon transplantation. In my view, either of these facts would inescapably lead the ordinarily skilled artisan to conclude that transplantation of such neural stem cell cultures would have a reasonable expectation of success in providing a therapeutic benefit to the host.

A. Transplantation of Neural Stem Cell Progeny According to the Claimed Methods For Delivery of Cellular Products Provides a Therapeutic Benefit

11. Applicants' neural stem cell cultures have been shown to be a useful tool for delivery of secreted cellular products which provide a therapeutic benefit when transplanted into the host. I draw the Examiner's attention to three publications that demonstrate that transplantation of cultures of neural stem cells that have been genetically modified to secrete nerve growth factor ("NGF"), according to the claimed methods, provides a therapeutic benefit.

12. Andsberg et al., "Amelioration Of Ischaemia-Induced Neuronal Death In The Rat Striatum By NGF-Secreting Neural Stem Cells", Europ. J. Neuroscience, 10, pp. 2026-2036 (1998) (copy attached as Ex. 2) reports that transplantation of NGF-secreting neural stem cell cultures into the adult rat striatum following middle cerebral artery occlusion ameliorated the death of striatal projection neurons that would have otherwise died due to the ischaemic insult. This clearly demonstrates a therapeutic benefit of the claimed methods.

13. Carpenter et al, "Generation and Transplantation of EGF-responsive Neural Stem Cells Derived From GFAP-hNGF Transgenic Mice", Exp. Neurology, 148, pp. 187-204 (1997) (copy attached as Ex. 3) reports that transplantation of NGF-secreting neural stem cell cultures into the adult rat striatum produced dense sprouting of p75 neurotrophin receptor-positive fibers

emanating from the underlying basal forebrain – a significant morphological change compared to controls, which I believe would be considered a therapeutic benefit in hosts where such neuronal regeneration and sprouting were desired. I note that the claimed methods were used. The authors conclude that "[t]he use of neural stem cells for transplantation into the CNS offers a number of advantages over transplantation of primary tissue or other cell lines." *See* p. 202. This to me also clearly demonstrates a therapeutic benefit of the claimed methods.

14. Kordower et al., "Grafts of EGF-responsive Neural Stem Cells Derived From GFAP-hNGF Transgenic Mice: Trophic and Tropic Effects in a Rodent Model of Huntington's Disease", J. Comp. Neurol., 387, pp. 96-113 (1997) (copy attached as Ex. 4) reports that intrastriatal transplantation of NGF-secreting neural stem cell cultures into an adult rat model of Huntington's disease (the well accepted and widely used quinolinic acid lesion model) resulted in sparing of striatal neurons immunoreactive for glutamic acid decarboxylase, choline acetyltransferase, and neurons histochemically positive for nicotinamide adenosine diphosphate. In addition the NGF-secreting transplants produced robust sprouting of cholinergic fibers from subjacent basal forebrain neurons. I note that the claimed methods were used. The authors conclude that "[t]hese data indicate that cellular delivery of hNGF by genetic modification of stem cells can prevent the degeneration of vulnerable striatal neural populations, including those destined to die in a rodent model of HD and supports the emerging concept that this technology may be a valuable therapeutic strategy for patients suffering from this disease." *See* p. 96. In my view, the authors clearly demonstrated a therapeutic benefit and clearly expressed their view that the results in the model are predictive of the human condition. For this reason, these data also clearly demonstrates a therapeutic benefit of the claimed methods.

B. Transplantation of Neural Stem Cell Progeny According to the Claimed Methods For Tissue-Specific Differentiation Provides a Therapeutic Benefit

15. Applicants' neural stem cell cultures have been shown to be useful tool for tissue-specific differentiation to provide a therapeutic benefit when transplanted into the host. I draw the Examiner's attention to several recent publications that demonstrate that transplantation of cultures of neural stem cells provide such a therapeutic benefit.

16. Qu et al., "Human Neural Stem Cells Improve Cognitive Function of Aged Brain", Ageing, 12, pp. 1127-1132 (2001) (copy attached as Ex. 5) report that when human neural stem cells were transplanted into aged rats (about 24 months old), according to the claimed methods, the cells not only survived, but also retained their multipotency and migratory ability. The results show that the human neural stem cells not only successfully differentiated into neurons and astrocytes, but importantly "both neurons and astrocytes migrated into the cortex and hippocampus in a well-defined and organized pattern in the brain." *See* p. 1132. Finally, the results demonstrate significantly improved cognitive function (in the standard and well accepted Morris water maze model). In my view this clearly demonstrates a therapeutic benefit of the claimed methods.

17. Akiyama et al., "Transplantation of Clonal Neural Precursor Cell Derived From Adult Human Brain Establishes Functional Peripheral Myelin in the Rat Spinal Cord", Exp. Neurol., 167, pp. 27-39 (2001) (copy attached as Ex. 6) reports that human neurosphere cultures (*i.e.*, an expressly disclosed embodiment of the neural stem cell cultures of this invention) when transplanted (according to the claimed methods) into a demyelinated adult rat spinal cord produced extensive remyelination with a peripheral pattern similar to Schwann cell myelination characterized by large cytoplasmic and nuclear regions, a basement membrane, and P0 immunoreactivity.[1/] Importantly, "the remyelinated axons conducted impulses at near normal

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1/ I note that the authors refer to a paper describing culturing the neurosphere cultures that was written by Dr. Joseph Hammang, one of the inventors (*see* reference 20), which confirms that the cells used here are cells of this invention.

conduction velocities". *See* p. 27. In my view this clearly demonstrates a therapeutic benefit of the claimed methods.

18. Kurimoto et al., "Transplantation of Adult Rat Hippocampus-Derived Neural Stem Cells into Retina Injured By Transient Ischemia", Neuroscience Letters, 306, pp. 57-60 (2001) (copy attached as Ex. 7) reports transplantation of rat neural stem cell cultures into the eyes of adult rats that underwent ischemia-reperfusion injury. The *in vivo* retinal ischemia-reperfusion model is a standard (and well accepted) experimental model that has been used to investigate the damage to the retina induced by transient ischemia. The authors report that in the eyes with the ischemia insult, the intravitreally injected neural stem cells invaded the retinal ganglion cell layer within a week of the transplantation and were identified in the retinal inner nuclear layer two weeks after the transplantation. At four weeks the donor cells were integrated into the host retina and expressed Map2ab, which indicated that the cells had differentiated into mature neurons. By comparison, in the control, none of the transplanted cells migrated to the retina. The authors conclude that "neuronal stem cells are good candidates to reconstruct the neural circuitry of ischemic injured retina, and show the potentiality of therapeutic transplantation using neuronal stem cells on retinal impairments that are generally regarded as incurable." *See* p. 59. I conclude from this that, both the authors (and myself) believe that this clearly demonstrates a therapeutic benefit of the claimed methods.

19. Likewise, Nishida et al., "Incorporation and Differentiation of Hippocampus-Derived Neural Stem Cells Transplanted in Injured Adult Rat Retina", Investigative Ophthalmology & Visual Science, 41, pp. 4268-4274 (Dec 2000) (copy attached as Ex. 8) report that transplantation of neural stem cells into mechanically injured adult retina results in incorporation and subsequent differentiation of the grafted stem cells into neuronal and glial lineages. Importantly, the authors conclude that "[n]eural stem cells are expected to be useful clinically for replacing damaged neurons or for ex vivo gene therapy." *See*, p. 4271. This statement confirms my similar statements throughout this declaration, and I believe is reflective of the general opinion of those of ordinary skill in the art.

20. Finally, I draw the Examiner's attention to several additional publications that, in my view, clearly demonstrate that the art is of the view that the claimed transplantation methods would provide a therapeutic benefit. In particular, *see e.g.* Ourednik et al., Novartis Foundation Symposium 231, Pub. John Wiley & Sons, Ltd. (2000) (Ex. 9), which is titled "Neural Stem Cells Are Uniquely Suited For Cell Replacement and Gene Therapy in the CNS". This title alone captures the sentiments expressed throughout my declaration. *See* also Vescovi et al., "Isolation and Intracerebral Grafting of Nontransformed Multipotential Embryonic Human CNS Stem Cells", J. Neurotrauma, 16, pp. 689-693, p. 689 (1999) (Ex. 10), which states "the use of human embryonic CNS stem cells should provide a reliable solution to some of the major problems that pertain to this field ...".

21. It is my firm belief, based on the literature and my own experience, that transplantation of such neural stem cell cultures according to the claimed methods would provide a therapeutic benefit to the host.

22. For all the foregoing reasons, I believe that the Examiner should withdraw the rejection and allow the pending claims.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Nobuko Uchida, Ph.D.

Signed at Palo Alto, California
this 30th day of July, 2001